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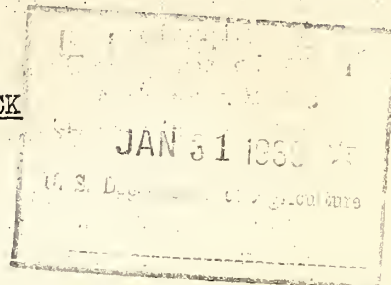
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Bureau of Animal Industry - Animal Husbandry Division

ARTIFICIAL INSEMINATION OF LIVESTOCK

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Increasing use of artificial insemination of livestock in Russia and other European countries during the last few years has created a great interest in this subject in the United States. While the method is not new, many improvements in technical details have been made in recent years and the method has been demonstrated to be an effective means for bringing about conception. It has been used extensively in the large breeding centers in Russia and is being used in cooperative dairy associations in Denmark and elsewhere. Since May 1938 four cooperative dairy breeding associations have been organized in New Jersey and New York in which artificial insemination is being used exclusively as a means of obtaining the services of a proved sire for the members of the associations. The method, however, has certain limitations and it is well that these limitations as well as its advantages be generally appreciated.

Uses for Artificial Insemination

The following are ways in which artificial insemination has proved useful in practical and experimental animal breeding: (1) by increasing the use of valuable proved sires; (2) to increase the percentage of conceptions; (3) by extending the period of usefulness of valuable sires, that because of age or crippling are unable to serve, or if able, can breed only a limited number of females; (4) where differences in size make natural insemination difficult or impossible; (5) in certain instances, as an effective aid in disease control; (6) to effect conception in valuable females that fail to conceive following copulation, or in females that refuse to accept the male; (7) to make use of young sires before they have attained sufficient size to mate normally or sufficient age to be bred to a large number of females; and (8) to bring about species crosses where natural mating is difficult or impossible.

While artificial insemination is a promising and often a very useful tool for breeders, it should be emphasized that it be used only by skilled workers, such as veterinarians or others who have been properly trained in the use of the method. A practical knowledge of the structure of the reproductive organs and of reproductive physiology is necessary, as well as a knowledge of the procedures to be followed in the collection of semen and insemination of the female. Certain precautions are also necessary to avoid injury to the breeding animals and to prevent spread of disease. In some species relatively little research has been done on this subject and until more information is available artificial insemination will not be entirely satisfactory for these species. If artificial insemination is to be used most effectively, satisfactory methods for the

collection of semen and the insemination of the female must be available and the stage of the estrual cycle of the female must be known so that insemination can be made at the proper time before ovulation. In general, it is not practicable at present for the average breeder but in certain cases, as in large breeding establishments, organized breeding associations, and experimental herds, it is both practicable and useful.

From the standpoint of livestock improvement its chief advantage over natural breeding lies in its possibilities for spreading the services of outstanding sires. Its use in applied breeding programs should be largely confined to such purposes although it can sometimes be used to very good advantage in increasing fertility in breeding herds.

Where properly used the danger of spread of disease is no greater and perhaps not so great as in natural breeding. However, this danger exists and certain precautions must be taken in the handling of equipment and apparatus, and to see that sires to be used are free from infectious disease that might be transmitted through the semen.

#### The Collection of Semen

Various methods have been and are being used for collection of semen but certain of these are unsatisfactory and are being replaced by newer and more satisfactory methods. The methods in most general use at this time for collection of semen are: (1) from the vagina following natural service; (2) by means of the artificial vagina; (3) by means of the breeder's bag in case of stallions; and (4) by massage in case of the bull, the chicken, and the turkey. In sheep induction of ejaculation by means of electrical stimulation has proved successful but it is primarily a laboratory method. Other means of collection now obsolete are: the sponge method; use of the sperm collector; and pan collection following withdrawal after coitus.

Collection from the vagina involves the aspiration of the semen from the floor of the vagina following service. This method is quite useful but has a number of objectionable features. The quantity of the semen obtained from one service is small and the semen is mixed with the secretions of the vagina. One of the most objectionable features is the danger of spreading disease, and where this method is used the greatest precautions should be taken to insure that the females used for collection are free from any infective reproductive diseases or disturbances. It is best to use females out of heat, and under no conditions should a female be used following parturition.

The artificial vagina has proved satisfactory for collection with most species on which it has been tried. The device consists of an outer tube of hard rubber or metal and a soft rubber inner tube. Warm water, 3 or 4 degrees above body temperature, is introduced into the space between the inner and outer walls until the desired pressure is obtained. The semen is collected in a small flask attached to one end of the inner tube. With this method of collection the semen is obtained in a near-sterile condition and the entire ejaculate is collected. Most animals may be easily trained to



use the artificial vagina.

The breeder's bag is a useful and satisfactory means for collection from the stallion but not all stallions will work with it. With it a large volume of ejaculate is obtained in an undiluted state. Collection by means of massage is an effective means of collection from the bull but considerable skill is required on the part of the operator if successful collections are to be made. Further, collections are difficult from some bulls. With an experienced operator large quantities of semen may be obtained in a nearsterile condition. In the chicken and turkey collections are easily made by an experienced operator and relatively large quantities of semen can be obtained at frequent intervals.

### The Examination of Semen

Certain characteristics of semen are indicative of the fertility of a male, so where a sire is to be used extensively for either natural breeding or artificial insemination careful examination of the semen should be made, both before the beginning of the breeding season and at weekly or two-week intervals during the breeding season. The factors to be considered in such an examination are motility, number of sperm, abnormalities of the sperm, quantity of ejaculate, color and consistency of ejaculate, the hydrogen-ion concentration, and freedom from bacteria, parasites, or cellular debris that would indicate a pathological condition of the genitals of the sire. This examination should not be confined to one ejaculate since considerable variation may occur in certain of these characteristics from time to time.

No one characteristic of the semen can be taken as an absolute index of fertility. Large numbers of highly motile sperm should be present in the ejaculate, the number of abnormal sperm should be low, and the color, consistency, and hydrogen-ion concentration of the semen should be normal for the species. If large numbers of inseminations are to be made it is also desirable that large quantities of semen are produced. If excessive numbers of bacteria or other abnormal cells are present, the male should not be used until the cause for this trouble is ascertained and the condition is corrected. In the case of cattle, examination should always be made for trichomonads, which may be transmitted through the semen. Examination for disease and parasites should best be made by a competent veterinarian.

### Insemination

Regardless of the species with which one is working, certain procedures and practices must be carefully followed if the greatest success is to be had. Before undertaking insemination or collection of semen it is necessary that proper apparatus be on hand, that it be clean, free from harmful chemical substances or bacteria, and completely dry. The instruments should be placed on a clean towel on a solid but movable table convenient to the operator, and they should be kept covered with a clean towel free from dust. The hands of the operator should be thoroughly washed with soap and hot water, then rinsed and dried preceding any collections or inseminations.

The paddock or quarters should be ample in size and free from any objects that might frighten or injure breeding animals. Where possible the same paddock should always be used for service since males work best in familiar surroundings and they soon learn to anticipate service when led into these quarters. Inseminations should be made in clean, dust-free quarters in order to prevent undue exposure of the vaginal mucosa of the female to infectious agents.

A very important consideration to the success of inseminations is to time the inseminations so that they will be made shortly before ovulation, for after ovulation eggs soon lose their capacity for fertilization; also, the duration of life of sperm in the female reproductive tract is quite short. In general, ovulation occurs near the end of estrus although some variation exists between different animals as well as in the duration of estrus and the intensity of manifestation of heat. Since it is often impossible for the breeder to determine the exact time of the onset of estrus, the following times are suggested for breeding or insemination:

Bitch: On the eleventh to thirteenth day after beginning to bleed.

Cow: Preferably twice, once shortly after beginning of heat and again 12-20 hours after beginning; if but once, 10-12 hours after onset of heat.

Ewe: During the last half of the heat period, or, if feasible, at 12-hour intervals so long as in heat.

Goat: During the last half of the heat period.

Mare: On the second and fifth day of heat if possible, but if to be bred only once, on the third day of heat. Where a mare is in heat three days after breeding, breed a second time.

Sow: Late on the first day or on the second day of heat, preferably the second day.

While relatively few experimental data are available, such experiments as have been made indicate that the best results with artificial insemination are obtained by introducing the semen well into the cervix or with some species directly into the uterus. In making inseminations the vagina should be opened with a speculum and the semen introduced with a syringe directly into the cervix, which can be seen at the anterior end of the vagina. Since spermatozoa are easily injured by coming in contact with most metals, a syringe with glass barrel and plunger should be used. In horses inseminations are frequently made with capsules. Where capsules are used the hand of the operator is inserted into the vagina and the capsule is pushed through the cervix. In case the cervix is not relaxed sufficiently to permit insertion of the capsule, relaxation may generally be obtained by massaging the cervix gently with the fingers.

The quantity of semen needed for successful fertilization is somewhat debatable, it being dependent upon such factors as the quality of the semen, the state of the reproductive tract of the female, the region into which the semen is introduced, and the stage of estrus. In the cow doses of 0.5 to 2 cc seem to be sufficient if placed in the cervix, while in sheep doses of 0.1 to 0.2 cc



are effective. In the mare doses of 15-25 cc should be used, while in the sow 50 cc of semen that has been freed of gelatinous particles seems sufficient. In the chicken it has been shown that insemination with doses of 0.05 cc of semen once a week results in fertility as high as that obtained from natural matings.

### Storage and Transportation of Semen

Where inseminations are to be made some time after collection certain precautions must be taken to keep the semen in a high state of viability. Temperature shocks must be avoided and it is best to protect the semen as much as possible from contact with air in order to decrease activity of the sperm. In addition, contact with metals, water, or harmful chemical agents must be scrupulously avoided. Where inseminations are to be made within one hour after collection, the semen may be placed in a small vial which has been thoroughly cleansed and dried. After being stoppered, the vial may be left at the temperature of a warm room (80°-85°F.) but should be set in a dark place and under no conditions be exposed to direct sunlight. Where semen is to be kept for longer periods it should be placed in a small vial immediately after collection, covered with sufficient high-grade neutral paraffin oil to exclude all air from the vial after it is stoppered, then wrapped in two thicknesses of paper and set in the refrigerator; or the paper-wrapped vial may be covered with two rubber thumb stalls and dropped in a vacuum bottle containing water at 37°-42°. This treatment provides for gradual cooling and maintenance at this temperature. Upon removal of the semen from the refrigerator or vacuum bottle, the temperature should be raised gradually before inseminations are made. This technique has been successful with bull, ram, and goat semen, but for the more watery types of semen (stallion and boar) satisfactory methods for storage have not been perfected.

During the last few years several successful long-distance shipments of bull and ram semen have been made for use in distant herds. Where artificial insemination is to be used in this way a close coordination of effort is necessary between the party making the collections and the one doing the inseminations. The shipments must be planned so that a minimum of time will elapse between collection and insemination and so that the semen will be on hand when the females are in the proper stage of estrus. Such use of artificial insemination is still in the experimental stages. Because of the difficulties involved it is probable that this use of artificial insemination will be practicable only in exceptional circumstances even though satisfactory methods for shipment are devised.

### Dilution of Semen

The main purpose of dilution is to spread the services of any given sire and is necessary only where a large number of females are being inseminated with the semen of a male. In Russia dilutors have been used extensively at the insemination centers with very good results. A good diluting agent must have the following qualities: (1) it must not be toxic to spermatozoa; (2) the osmotic relations must be similar to those existing in the undiluted semen; (3) the pH of the semen must be favorable for continued viability of the spermatozoa; (4) it should contain a buffering solution to protect against marked changes in the pH; and (5) it should be inexpensive and easy to prepare.

Where good dilutors are used the volume of semen may be increased several-fold without decreasing the percentage of conceptions. When used they should be added to the semen slowly and the tube containing the semen should be shaken while the dilutor is being added.

Various diluting solutions have been developed. The SGC2 formula devised by Russian workers is a satisfactory diluent for bull semen. It is made as follows:

Distilled water	1000.0 cc
Sodium Sulphate ( $\text{Na}_2\text{SO}_4$ )	13.6 grams
Glucose	12.0 grams
Peptone	5.0 grams

At the University of Minnesota the following formula has been found satisfactory for sheep:

Distilled water	1000.0 cc
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	5.4 grams
$\text{KH}_2\text{PO}_4$	3.2 grams
$\text{CaSO}_4$	Saturate

These solutions should be adjusted to get a pH of about 7.

#### Other Considerations

An important factor in all breeding programs, whether natural breeding or artificial insemination is used, is to have the male in the best possible breeding condition. The management program, if possible, should be such that males and females are improving in condition throughout the breeding season. The ration of the male should be well balanced, containing ample supplies of minerals, carbohydrates, proteins, and vitamins. Excessive fatness in breeding males should be avoided for the fertility of such males is usually lowered by the production of fewer sperm or sperm of lower quality.

Where summer temperatures are frequently above  $80^{\circ}$ - $85^{\circ}\text{F}$ . it is good practice to allow stud males access to cool quarters, cool water, and ample shade. In rams and several laboratory species it has been definitely shown that high atmospheric temperatures lower the sperm production and result in a high count of abnormal sperm.

If males are to be used for artificial insemination, it is important that they be docile, well broken to lead, and free from bad habits. Most males may be easily trained to mount females out of heat or to mount dummies.

Much work has been done in an attempt to evaluate the influence of frequency of service on reproductive efficiency. Much variation exists between individual males in this respect and between different species. For those species that produce large quantities of ejaculate at each service (stallion, boar, dog) the sperm number per ejaculate decreases rapidly, while in species in which the ejaculate is small a great many matings may be made in a relatively short time without greatly decreasing the number of sperms per ejaculate or their viability. In the boar one mating a day seems sufficient,



While in rams it would appear that more than three matings may be made per day without serious depletion of the sperm number. In many mature stallions from two to five matings per day may be made without seriously depleting the sperm supply but good management would seem to call for lighter use following days with such heavy breeding schedules. In the bull two matings a day may be made over prolonged periods without seriously depleting the sperm supply.

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